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RESPONSES OF LEAF SPECTRAL REFLECTANCE TO PLANT STRESS[1]

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Leaf spectral reflectances were measured to determine whether leaf reflectance responses to plant stress may differ according to the agent of stress and species. As a result of decreased absorption by pigments, reflectance at visible wavelengths increased consistently in stressed leaves for eight stress agents and among six vascular plant species. Visible reflectance was most sensitive to stress in the 535-640-nm and 685-700-nm wavelength ranges. A sensitivity minimum occurred consistently near 670 nm. Infrared reflectance was comparatively unresponsive to stress, but increased at 1,400-2,500 nm with severe leaf dehydration and the accompanying decreased absorption by water. Thus, visible rather than infrared reflectance was the most reliable indicator of plant stress. Visible reflectance responses to stress were spectrally similar among agents of stress and species.

Within the 400-2,500-nm wavelength range, which includes most of the incident solar spectrum (Gates, 1980), the spectral reflectance of vegetation may indicate plant stress. Leaf reflectance responses to environmental conditions that inhibit growth generally involve increased reflectance in the visible (380-760-nm, Rossotti, 1983) or infrared (760-2,500-nm) spectra. Such reflectance increases have been reported in response to agents of stress that are biological (e.g., Ahem, 1988) as well as physicochemical (e.g., Schwaller, Schnetzler, and Marshall, 1983) in origin.

Although leaf reflectance has been studied in response to numerous stress agents (see Carter et al., 1992 for additional review), the spectral regions, or wavelengths, at which leaf reflectance is most responsive to stress remain largely undefined. Also, the extent to which particular stress agents yield spectrally unique leaf reflectance responses has not been established (Jackson, 1986). Thus, the purpose of this paper was to: 1) summarize leaf reflectance responses for a variety of stress agents and among several species; 2) determine the wavelengths at which leaf reflectance is generally most responsive to stress; and 3) estimate the extent to which differing agents of stress may yield spectrally unique reflectance responses. Reflectance responses to stress were compared among four biological and four physicochemical stress agents using six vascular plant species.

MATERIALS AND METHODS

Leaf spectral reflectances were measured for stressed plants vs. relatively unstressed plants (controls) that were naturally or experimentally grown in the field. Responses to stress agents of biological origin were determined for plant competition in loblolly pine *(Pinus taeda* L.), powdery mildew disease in golden euonymus *(Euonymus japonica* variety Aureo-marginata), insufficient infection with ectomycorrhizal fungi in slash pine *(Pinus elliottii Engelm.), and senescence in live oak (Quercus virginiana* Mill.). Responses to stress agents of physicochemical origin were determined for exposure to herbicide (DCMU) in persimmon *(Diospyros virginiana* L.), increased atmospheric ozone in loblolly pine, the sandy soils and high salinity of a barrier island vs. mainland site in slash pine, and short-term dehydration in switchcane *(Arundinaria recta* [Walt.] Muhl.). Reflectance was measured for five replicates per treatment except in the cases of ectomycorrhizae in slash pine (N: 3) and ozone in loblolly pine (N= 6).

To include reflectance responses to competition, ozone, and the barrier island environment, data were extracted from earlier studies (Carter et al., 1989, 1992; G.A. Carter and D.R. Young, unpublished data, respectively). Methods to determine reflectance responses to dehydration also were described previously (Carter, 1991), and were used to compare leaf reflectances at 30% vs. 100% relative water contents (RWCs) in/1. *recta.* RWC was computed by subtracting leaf dry mass from fresh mass, multiplying the difference by 100, and dividing by the difference of fully turgid mass minus dry mass.

The reflectance response to a plant disease was determined for nonvariegated leaves of euonymus that were infected vs. uninfected with powdery mildew fungus (Fungi Imperfecti). Leaves were collected from infected shrubs that were approxi-mately 2 m in height and growing on a local sun-exposed site. The leaves were returned to the laboratory and cleaned using water and mild abrasion to remove fungal hyphae from the surface prior to reflectance measurements.

The effect of insufficient ectomycorrhizae on leaf reflectance was determined for slash pine seedlings that were inoculated or noninoculated with the beneficial mycorrhizal fungus *Pisolithus tinctorius* (Pers.) Coker and Couch. Canopy reflectances for the noninoculated vs. inoculated seedlings were compared and methods described previously (Cibula and Carter, 1992). During the earlier study, leaf reflectances for three seedlings per treatment were measured using bundles of approximately 200 needles per seedling and methods that were appropriate for pine needles (Carter, 1991).

Reflectance responses to evergreen senescence were determined for senescent (yellow) vs. nonsenescent (green)

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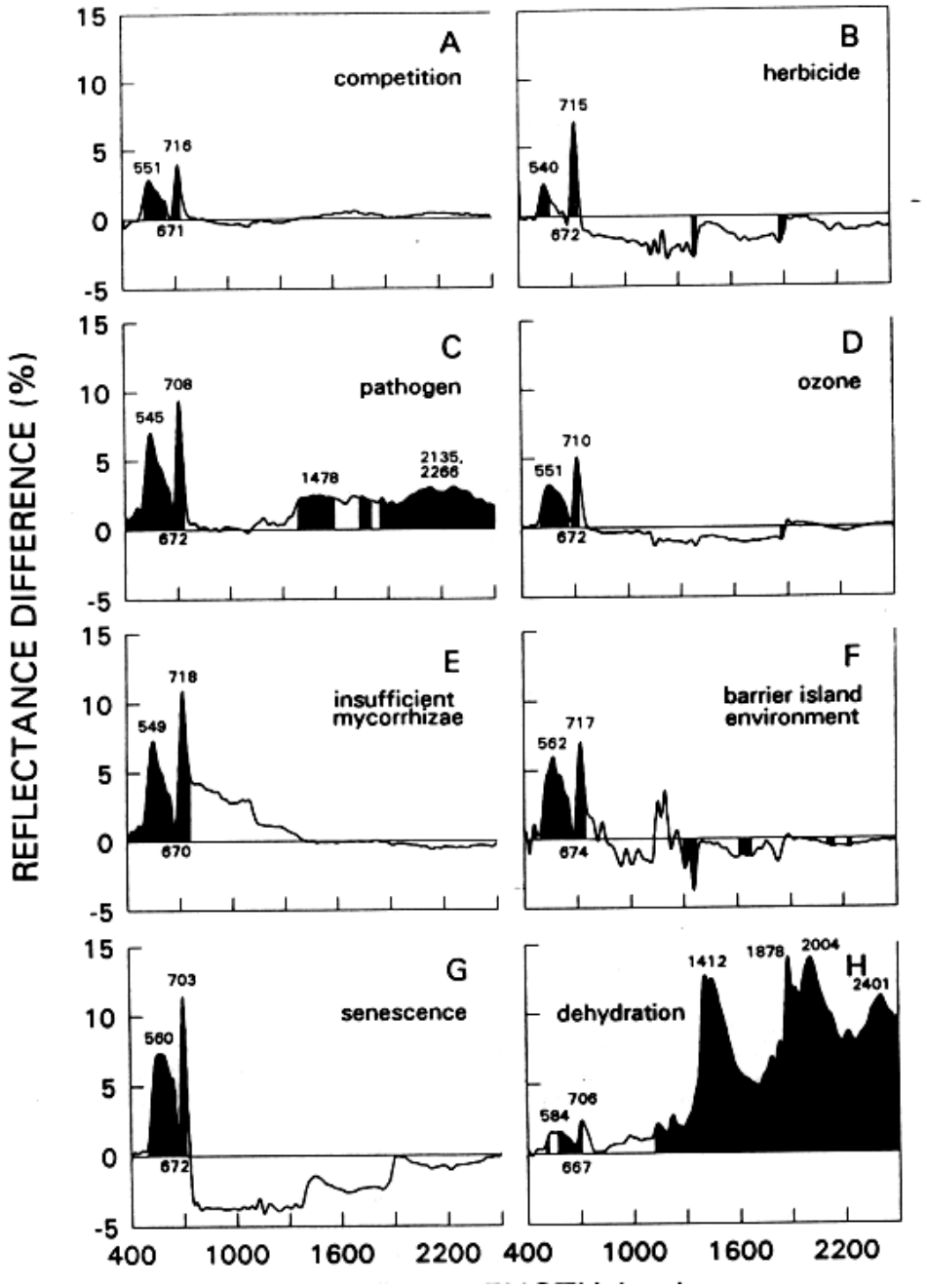

Fig. 1. Reflectance differences for stressed vs. nonstressed leaves throughout the 400-2,500-nm spectrum. Visible wavelengths are approximately within the 400-760-nm range. Differences were computed by subtracting the mean reflectance of nonstressed leaves (zero line) from that of stressed leaves. Means were for five replicates (N= 5) except in D (N= 6) and E (N= 3). Darkened regions indicate differences 'hat were significant (P -< *0.05)* according to ANOVA and Dunnett's test, and numbers inside a graph indicate wavelengths for difference maxima or minima. See Table I for exact wavelength ranges of the significant differences. Species represented are loblolly pine (A, D), persimmon (B), euonymus (C), slash pine (E, F'), live oak (G), and switchcane (H).

oak leaves that were collected locally from a mature tree growing in an open field. Senescence in live oak involves a color change from green through yellow to brown.

The reflectance response to damage induced by a herbicide was determined for persimmon seedlings that had established on a local abandoned roadside. Seedlings approximately 0.5 m in height were exposed to a single application to the soil of 8 g/liter DCMU powder (Diuron,

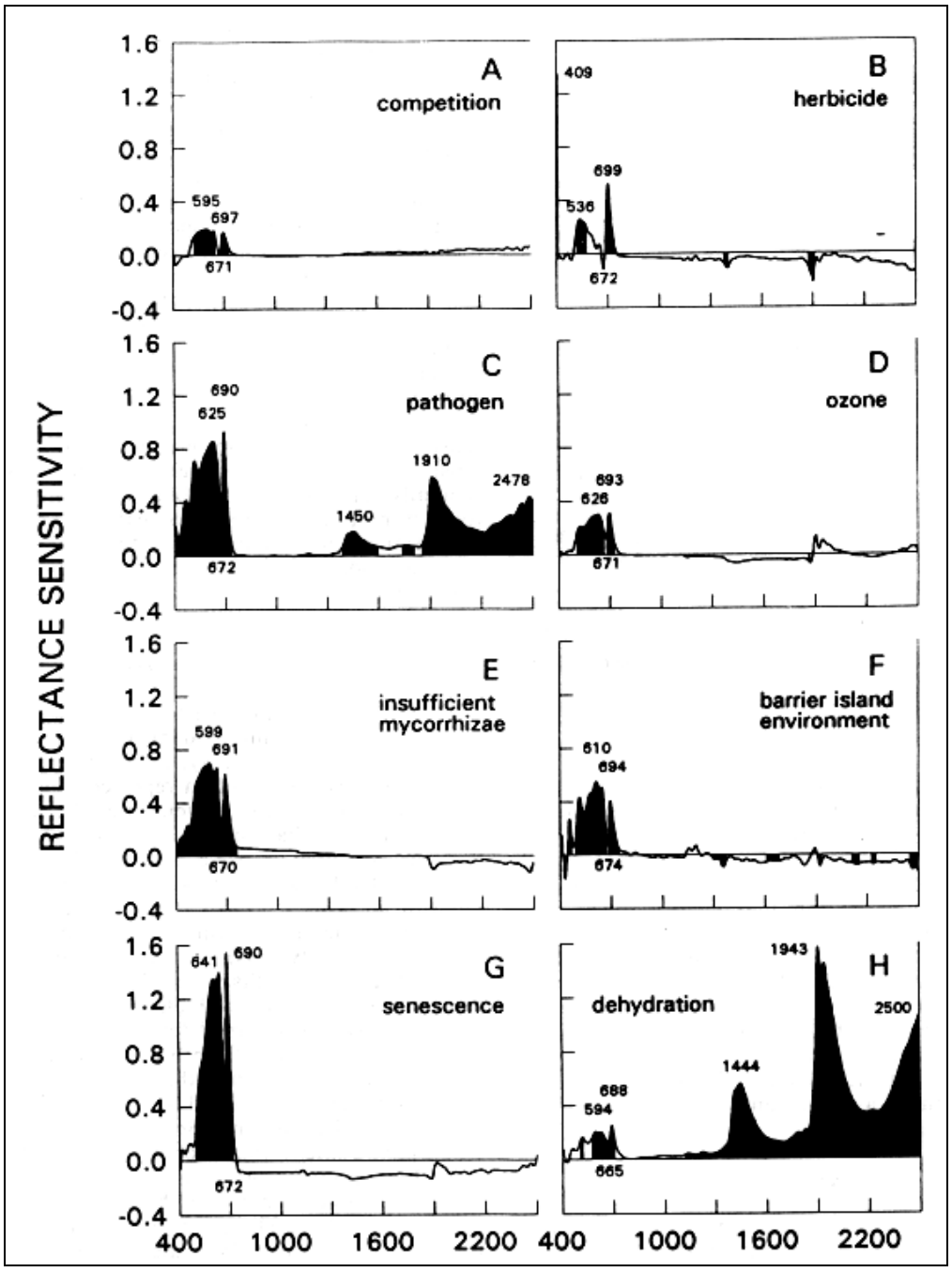

Fig. 2. Spectral sensitivity of leaf reflectance to plant stress. Sensitivities were computed by dividing the reflectance differences (Fig. 1) by reflectances of the nonstressed leaves (zero sensitivity). Darkened regions indicate sensitivities for which the reflectance difference (Fig. 1) was significant ($P \sim 0.05$) according to ANOVA and Dunnett's test, and numbers inside a graph indicate wavelengths of sensitivity maxima or minima. See Table 1 for exact wavelength ranges of the significant sensitivities. Species represented are loblolly pine (A, D), persimmon (B), euonymus (C), slash pine (E, F), live oak (G), and switchcane (H).

du Pont de Nemours, Wilmington, DE) in water. Leaf reflectances for herbicide-treated vs. untreated plants were measured 8 d following herbicide treatment.

For all agents of stress and species, reflectance was measured in the laboratory using a scanning spectroradiometer (IRIS model 11, GER, Inc., Milbrook, NY) and methods described earlier (Carter, 1991). Reflectances were obtained at each of 768 radiometer channels that were calibrated to wavelength using a krypton lamp and reflectance calibration standards (Labsphere, Inc., North

TABLE 1. *Wavelength ranges (nm) for significant reflectance differences and sensitivities*[a]

Stress	Wavelengths	Stress	Wavelengths
Competition	403–409	Herbicide	405–409
	525–650		519–573
	686–728		688–735
			1,384–1,401
			1,875–1,905
Pathogen	405–418	Ozone	506–661
	428–738		680–723
	1,390–1,595		1,865
	1,743–1,805		
	1,856–2.508		
Insufficient mycorrhizae	415–760	Barrier island	409–413
			420–436
			445–468
			491–667
			681–742
			1,298–1,373
			1,612–1,680
			1,916–1,927
			2,114–2,151
			2,230–2,245
			2,452–2,508
Senescence	498–715	Dehydration	506–519
			571–708
			1,119–2,508

[a]Reflectances of stressed leaves were significantly different ($P \leq 0.05$) from those of nonstressed leaves in the specified wavelength ranges according 10 ANOVA and Dunnett's test. Difference and sensitivity values arc obtained from Figs. 1 and 2, respectively.

Sutton, NH). Spectral resolution was 1-2 nm in the 4001,113-nm range, 3-4 nm in the 1,136-1,894-nm range, and 5-6 nm in the 1,900-2,508-nm range.

For each agent of stress, reflectance differences were computed by subtracting the mean leaf reflectance of relatively nonstressed leaves from that of stressed leaves at each spectroradiometer channel (Carter, 1991). Significance ($P \leq 0.05$) of the reflectance difference at each channel was determined by ANOVA and Dunnett's means comparison (Steel and Tome, 1960) to the unstressed controls. Reflectance sensitivity at a given wavelength was computed by dividing the reflectance difference by the control reflectance at each channel (Carter, 1991). Sensitivities were defined as significant if the associated difference was significant.

RESULTS

Visible reflectance, particularly in the green spectrum (491-575 nm) near 550 nm and red spectrum (647-760 nm, Rossotti, 1983) near 710 nm, increased consistently in response to stress regardless of stress agent or species (Fig. 1; Table 1). Differences near 710 nm were greater than those near 550 nm. With dehydration, a peak difference occurred in the yellow spectrum (575-585 nm, Rossotti, 1983) at 584 nm (Fig. 1H). Differences in the visible spectrum generally were least at violet (380-424nm) and blue (424-491-nm, Rossotti, 1983) wavelengths. The exception was substantially increased reflectance near 409 nm in response to herbicide damage (Fig. 1 B; Table 1). Difference minima occurred also near 670 nm in all cases.

Infrared reflectance generally did not change with stress or changed inconsistently. As exceptions, infrared reflectance increased substantially with fungal infection in euonymus leaves (Fig. *I C),* and particularly with dehydration to 30% RWC in switchcane (Fig. 1 H). Peak differences accompanying dehydration occurred near 1,400 nm, 1,900 nm, 2,000 nm, and 2,400 nm (Fig. 1 H).

Reflectance sensitivity (Fig. 2) indicates the wavelengths at which a linear response detector, such as photographic film, would most likely detect a reflectance response to plant stress (Cibula and Carter, 1992). Sensitivities were generally greatest in the orange (585647-nm, Rossotti, 1983) and red spectra, except for peaks in the violet and green spectra that accompanied herbicide damage. These maxima occurred within the 535-640-nm and 685-700-nm ranges except for the maximum near 409 nm in herbicide-damaged persimmon. In most cases, sensitivity at 685-700 nm was greater than or equal to sensitivities at shorter wavelengths. Sensitivities were generally least in the violet-blue spectrum, and a sensitivity minimum occurred also near 670 nm in all cases. Dehydration in switchcane and fungal infection in euonymus yielded sensitivity maxima near 1,450 nm, 1,9001,950 nm, and 2,500 nm.

DISCUSSION

The constancy of increased visible reflectance as a response to stress was quite evident among the various stress agents and species. Notably, increased reflectance near 700 nm represents the often reported "blue-shift"; i.e., the shift toward shorter wavelengths of the red-infrared transition curve that occurs in stressed plants when reflectance is plotted vs. wavelength (Horler, Dockray, and Barber, 1983; Rock, Hoshizaki, and Miller, 1988; Curran, Dungan, and Gholz, 1990; Cibula and Carter, 1992). The reflectance difference maxima near 550 nm and 710 nm, and the sensitivity maxima near 620 nm and 700 nm, occurred at wavelengths where the absorptivity of chlorophyll a is relatively low (Hoff and Amesz, 199 l). With low absorptivity, even small decreases in chlorophyll content could result in significantly decreased absorption and increased reflectance. Thus, these difference and sensitivity maxima can be explained by stress-induced decreases in chlorophyll a content. However. small decreases in chlorophyll content would not increase reflectance greatly in the blue spectrum and near 670 nm because of the high absorptivity of chlorophyll in these spectral regions (Hoff and Amesz, 1991). Thus, difference and sensitivity minima occurred consistently in the blue spectrum and near 670 nm.

Visible reflectance responses to stress generally were not unique for a given stress agent, lending support to the view that plant physiological responses to stress are similar

regardless of the cause of stress (Chapin, 1991). With plant stress, major reflectance differences occurred generally in the green and red spectra, and major sensitivity peaks occurred generally in the orange and red spectra. The importance of subtle spectral differences in peak responses to stress (e.g., Figs. 1,2) remains undetermined. These might be explained by differences in pigment metabolism induced by different agents of stress, but it seems probable that they result from variables such as inter-specific differences in pigmentation.

The prominent sensitivity maxima in the water absorption bands near 1,450, 1,940, and 2,500 nm thai occurred with fungal infection in euonymus and dehydration in switchcane were characteristic of decreased absorption by leaf internal water (Carter, 1991). Similar maxima might have occurred in response 10 the other stress agents also if reflectances had been measured later during the physiological responses to stress. Reflectance in the water absorption bands would be expected to increase in any leaf following stress-induced damage as a result of dehydration (e.g., Carter et al., 1992).

To conclude, increased reflectance in the visible spectrum is the most consistent leaf reflectance response to plant stress. Infrared reflectance responds consistently only when stress has developed sufficiently to cause severe leaf dehydration. Results suggest that leaf spectral reflectance is most likely 10 indicate plant stress in the sensitive 535640-nm and 685-700-nm wavelength ranges. Photography or digital imaging within these spectrally narrow ranges (e.g., Cibula and Carter, 1992) may provide improved capability to detect plant stress not only in individual leaves, but for whole plants and densely vegetated landscapes.

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